Feeding stimulants from the camphor tree leaves (*Cinnamomum camphora* Sieb) to the green-banded swallowtail (*Graphium sarpedon* L.) larva.

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Abstract
Feeding stimulants for larva of the green-banded swallowtail (*Graphium sarpedon* L.) from the camphor tree (*Cinnamomum camphora* Sieb) were investigated. Four compounds, chlorogenic acid, sucrose, quercetin 3-β-*O*-glucoside and octadecatrienoic acid were identified from the methanol extract of *C. camphora* leaves. The mixture of the three compounds (chlorogenic acid, sucrose and quercetin 3-β-*O*-glucoside) showed a strong feeding stimulus activity towards the larva, but they did not show any activity separately. On the other hand, octadecatrienoic acid acted as a feeding stimulant alone.

Introduction
Oviposition stimulants for adult female butterflies from their host plants have been well investigated in the Papilionidae, however there are only a few reports on feeding stimulants for butterfly larva from their host plants. We have been conducting the study on the host selection of the green-banded swallowtail (*G. sarpedon*) from the camphor tree (*C. camphora*) by investigating both of feeding stimulants and ovipositing stimulants. Here, we will report on the feeding stimulants for larva from the camphor tree.

Material and Method
Insect & Plant: Egg or larva of *G. sarpedon* were collected from camphor trees around the campus. The collected eggs or larvae was individually reared on fresh leaves of camphor tree (*C. camphora*) in a plastic Petri dishes at 25° C (16L:8D). Leaves of camphor tree were also collected in the campus.

Bioassay: A test sample solution (200μL, 1g leaf eq) was applied to a polystyrene foam disk of hemicycle of 25 mm in radius. Three to six piece of the polystyrene foam disks were place on the box as shown Fig.1 and introduced three larvae (late 2nd , 3rd or early 4th instar larva) and the box was covered with a sheet glass. They were allowed to feeding polystyrene foam disks for 24 H at 25° C (16L:8D). Feeding area was calculated by comparing of disks area before and after the test.

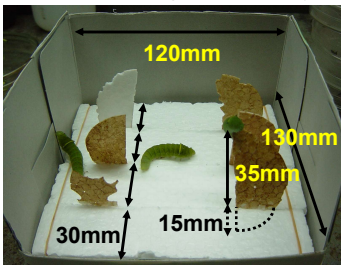

Fig.1 Bioassay apparatus

Chemicals: Purification of the active components was conducted by liquid-liquid partition, ODS column and reverse-phased preparative HPLC as shown in Fig.2. Isolated compounds were analyzed by NMR, LC-MS, GC-MS and UV spectra. Esterification of isolated fatty acid was conducting by using diazomethane.

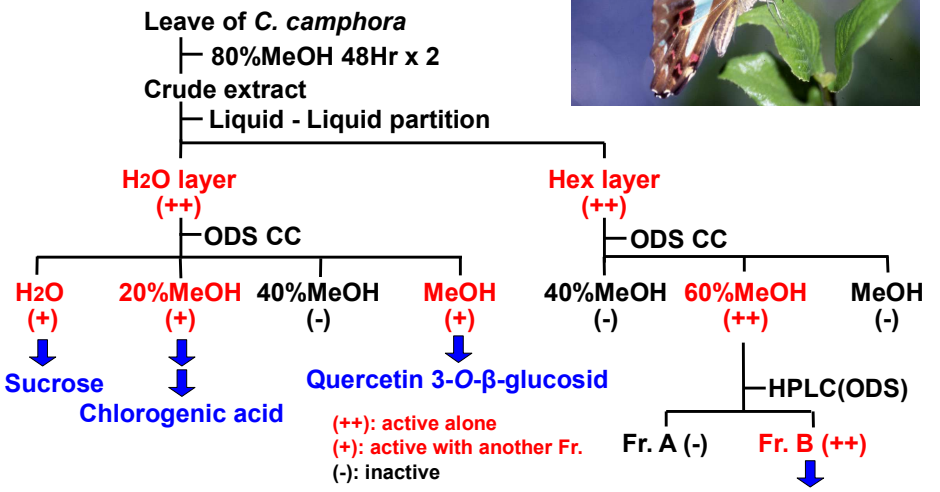

Fig.2 Isolation procedure of feeding stimulants

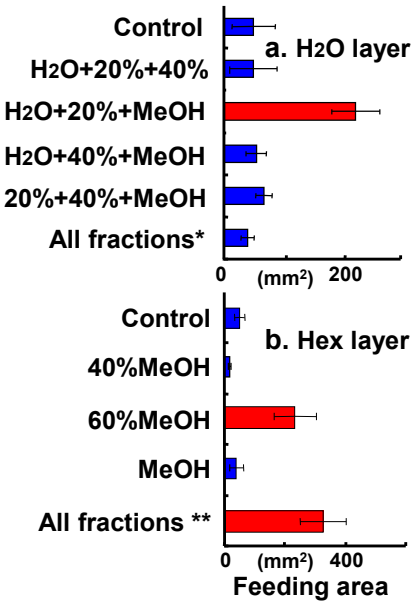

Fig.3 Activities of fractions
(a)Activities of combine fractions on the procedure of purification of water layer.
(b)Activities of fractions on the procedure of purification of hexane layer.
* H_2O+20%+40%+MeOH ** 40%+60%+MeOH

Result
Both of water and hexane layer, after liquid-liquid partition of the methanol extract, showed strong feeding stimulus activity. The water layer was purified with an ODS column, each fraction was not separately active, however, when H_2O Fr., 20%MeOH-H_2O Fr. and MeOH Fr. were mixed, the strong activity was recovered (Fig.3a). Then the three fractions were further purified by a HPLC. Consequently, sucrose, chlorogenic acid and quercetin 3-*O*-β-glucoside were isolated, and the mixture of the three compounds showed strong activity. The hexane layer was also fractionated by an ODS column and the activity was recovered in 60%MeOH-H_2O Fr.(Fig.3b) A fatty acid was isolated by a HPLC, and spectroscopic analysis indicated fatty acid to be octadecatrienoic acid, however double bonds positions and their geometry were unclear.

Conclusion
Three synergistically feeding stimulus compounds and a fatty acid were identified as feeding stimulants for larva of *G. sarpedon*. All compounds were generally occurring in plant, so it was thought that another factor(s) might play a important role for host selection of *G. sarpedon*. It would be needed to conduct further studies on their oviposition stimulant and attractant for adult butterfly.